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(From the Laboratory of Experimental Pathology of the University of Pennsylvania)

Reprinted from
THE JOURNAL OF MEDICAL RESEARCH, VOLUME XVII., No. 3
(New Series, Vol. XII., No. 3), pp. 299-319, December, 1907



BOSTON
MASSACHUSETTS
U.S.A.



ON THE DIFFERENCE IN THE RESULTS OBTAINED AFTER
INOCULATION OF TUMORS INTO THE INDIVIDUAL IN
WHICH THE TUMOR HAD DEVELOPED SPONTANEOUSLY,
AND INTO OTHER INDIVIDUALS OF THE SAME SPECIES.

LEO LOEB AND SAMUEL LEOPOLD.

*(From the Laboratory of Experimental Pathology of the University of
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The aim of the following investigation was threefold:

1. It is known that the large majority of all tumors found in dogs cannot be transmitted through transplantation to other dogs. The lymphosarcoma found in the genital region of dogs forms a marked exception to this rule; it is relatively easy to transplant. Thus far the experimental investigation of tumors has not concerned itself much with the question of conditions causing the nontransplantability of tumors. Is it caused through a low vitality of the tumor cells, which do not sufficiently resist the processes of excision, mincing, and inoculation? That such a possibility should be considered may be concluded from the fact that, even vigorously growing tumors which can be easily transplanted may, through physical and experimental interference, be so changed that they grow only weakly after transplantation (Loeb). Or, when they cannot be transplanted into other animals, is it due to the presence in another animal of the same species of substances which are injurious to their growth? In other words, in the latter case, is the chemical composition of the circulating body fluid not adapted to the life of the tumor cells of another animal of the same species?

2. A few cases are known in man, in which a tumor could be successfully transplanted into the same individual from which the tumor originated (cases of Hahn¹ and Cornil²). The transplanted pieces continued their growth in these cases. A few other cases are known in which tumors formed in man were inoculated into others (experiments of Alibert³ and Senn⁴). In these cases the results

were negative. Shall we conclude from their experiments that in human tumors pieces can be excised and reinoculated successfully into the same individual where they continue to grow, but that after the same operative interference, which perhaps means considerable injury to the tumor cells, the latter cannot be successfully transplanted into other individuals? The results of the few experiments quoted above suggest such a conclusion.

No experiments are recorded where in man the same tumor was simultaneously transplanted into the individual spontaneously affected with the tumor, and into other individuals. Such an experiment alone can decide this question.

A similar experiment one of us⁵ made about six years ago, in the case of an adenoma of the mammary gland in a white rat; in this case the tumor was successfully transplanted into the same rat, but not into other rats; in the latter it soon became necrotic.

We decided, therefore, to extend our former experiments by using for this purpose a mammary tumor found in a dog.

3. The mammary tumors of dogs are frequently mixed tumors, consisting of adenomatous tissue, and various connective tissue structures, such as myxomatous tissue, cartilage, hyaline, and ordinary connective tissue. Does a certain equilibrium exist between these different structures, which is changed after transplantation so that one tissue preponderates over another, or do they preserve their original relations?

In an old female spaniel, blind in one eye, two tumors were found; one situated in the left mammary gland, forming a large mass about 2×4 inches, nodular, the skin over it being adherent, though free at the sides and base. Over the upper portions there was an ulcer $\frac{3}{4} \times \frac{1}{2}$ inches. The tumor was firm and movable. The second tumor was situated on the opposite side, at the base of one of the middle mammæ. It consisted of several nodules, and was softer and whiter. Neither mass was encapsulated.

On October 25, 1906, the tumors were excised; part of the excised material was cut into small pieces, suspended in salt solution, and injected subcutaneously into the same dog

near the seat of the right tumor. Another injection with the tumor material was made intra-abdominally.

Another dog, likewise an old spaniel (dog No. 2), was injected in both flanks. A few small nodules of the tumor were not removed from dog No. 1 during the operation, but were left in place.

Ten days later, October 29, 1906, the injected particles of tumor material could be partly felt. The place where the tumor of the left side was situated was edematous.

November 5, 1906, the left side in the original dog showed the edematous swelling decreased. The wounds were healing. At the base of the tumor some nodules could be felt. Injected particles of tumor material could likewise be felt. In dog No. 2 the injected tumor material could also be felt.

November 10th, the transplanted nodules seemed to get somewhat smaller. In the operation wound of the original dog a few small nodules could be felt. The edema of the left side has decreased.

November 22d, in the original dog the transplanted pieces could be felt near the place where the old tumor was situated. In dog No. 2 the transplanted nodule could no longer be felt. The nodules in the scar of the original dog seemed to be slightly growing.

December 5th, the tumor nodules showed no growth for ten days, if anything they have decreased in size a little. This applies to the nodules not transplanted, as well as to the transplanted nodules in the original tumor dog. One nodule was removed for microscopic examination. One part of a transplanted nodule was excised from the dog with the original tumor. Another transplanted nodule was divided into two parts, and one retransplanted into the same dog. Through another nodule a silk thread was pulled, and the skin above the nodule closed by stitches. The second part of the nodule, which had been cut in two parts, was transplanted subcutaneously into the right side of a female fox terrier that was suckling her young (dog No. 3).

December 13, 1906, the wound made December 5th

showed good healing. Around the wound a few nodules could be felt. The piece transplanted the second time into the same dog, and the nodule from which a piece had been taken, showed no growth. The other small nodule showed no decrease in size.

December 18, 1906, the nodules in the mammary gland of the original tumor dog showed a little increase in size. In the suckling fox terrier a little nodule could be felt at the place where the transplantation had occurred thirteen days before.

December 29, in the suckling fox terrier the transplanted nodule had almost disappeared. In the original tumor dog the transplanted nodules were present, but showed no growth. Some showed a slight apparent increase.

December 31, 1906, small nodule removed from left, lower mammary gland of original dog, one portion of which was retransplanted subcutaneously over the right side of the thorax, near the costal margin. Another portion was transplanted to a black spaniel (dog No. 4) subcutaneously near the upper, right mammary gland. Tumor nodules of previous transplantations were excised and placed in Zenker's fluid.

January 3 and 5, 1907, the transplanted pieces in both dogs could be felt.

January 8, 1907, in the right, lower mammary gland of original tumor dog a small nodule could be felt.

January 9th, in dog No. 4 some enlargement noticeable at place of transplanted piece.

January 11th, transplanted piece from dog No. 4 was excised from microscopical examination.

January 18, wound in original tumor dog healed.

January 24, the piece transplanted December 31st in the original tumor dog was found to have decreased slightly in size.

January 28th, piece transplanted into original dog on December 31st was removed, and nodule in left mammary gland, which apparently had grown recently, was excised. After excision, the excised piece was found to contain less

tumor tissue than had been expected from palpation before operation. One part of the excised piece retransplanted in original tumor dog over sternum, another piece transplanted in dog No. 4 (black spaniel).

February 7, 1907, in dog No. 1 and No. 4 the transplanted nodules could not be felt. Perhaps they consisted of other than tumor tissue.

February 23d, tumor nodules could only be felt in the original tumor dog (No. 1). The nodules appeared to be stationary. In the other three dogs no nodules could be detected.

We can conclude from these observations: (1) that the pieces transplanted into three other dogs disappeared entirely in about three or four weeks after transplantation. About ten days after transplantation they may show a transitory apparent increase in volume. Microscopic examination reveals, however, that this apparent growth is due to a proliferation of connective tissue, which surrounds and invades the transplanted piece. The pieces did not grow in dogs that were very similar in relationship to the one in which the original tumor was found, neither did they grow in a dog which was suckling young and whose mammary glands were, therefore, in a condition of functional hypertrophy; (2) all the pieces remained alive in the dog originally affected with the tumor, and the periods at which the tumor nodules were transplanted did not cause any difference in that respect. The transplanted piece did neither materially increase nor decrease in size. Small changes, which were apparently found in the original tumor nodules as well as in the transplanted pieces, may perhaps have been due to differences in blood supply, or in some other functional condition; and (3) that either cutting out a piece from a tumor, or transplanting it a second time, or pulling a thread through a nodule, did not cause it to resume active proliferation; (4) it is to be noticed that the stationary condition of the tumor nodules affected equally the original as well as the transplanted nodules in dog No. 1.

Microscopical Examination.

I. Pieces of original tumor not transplanted:

1. Portions of ulcerated tumor taken out October 25, 1906. The tumor consists of numerous areas of proliferation, showing connective tissue and epithelial changes; some consist entirely, or almost entirely, of proliferating connective tissue, with transitions from the connective tissue, rich in spindle cells, to hyaline connective tissue. In other areas transitions are occurring in the following manner: (1) the glandular tubules become larger, and are filled with colloid material; (2) the arrangement of the tubules becomes more irregular, and the connective tissue around the tubules increases and shows hyaline, myxoid, and cartilaginous changes. Some of these areas are surrounded by a connective tissue capsule, at many places glandular tissue is found that resembles normal mammary gland, but in which, here and there, transitions into adenomatous tumor structure can be seen. Some of the peculiarities of the tumor will be indicated in the description of the following pieces:

Area A: A small area is present in which the glandular epithelium shows just beginning transitions into the abnormal. Slight dilatation of the tubules, containing small masses of colloid material.

Area B consists of gland tubules which are cystic and contain colloid masses in the center. Between the epithelial structures are large masses of connective tissue which are very cellular in character. The latter show hyaline and, in places, myxoid changes. In the connective tissue we find colloid masses which may perhaps represent the remains of necrotic glandular structures. Into the dilated ducts, intracanalicular growth is taking place, the connective tissue matrix of the ingrowth being supplied by myxomatous tissue. In some of the narrower tubules isolated cells carrying yellow pigment are found.

Area C: In another area the cystic dilatation is absent and the structure resembles the normal mammary gland. The areas B and C are separated from each other by a connective tissue capsule.

Area D: In this area more normal gland structures are noted; nearby adenomatous formations with papillomatous ingrowths are seen, and near this dilated cystic tubules are situated. Inside the latter we find cell nests with masses of golden pigment.

Area E: In this area we note the presence of normal breast structure. In places the pigment containing cells, and in some other spaces the colloid materials seem to be fused. The colloid masses are partly derived from the breaking down of the pigment cells. In some of the glandular structures blood can be found. The walls of the blood vessels are thickened.

Area F: Another section shows canals lined by cells, containing many vacuoles, which were probably filled by fat. Nearby we find intracanalicular growth. The connective tissue is more or less hyaline. Outside the capsule of this node we find an area consisting of connective tissue in which the intercellular substance has a myxoid character, and takes the hematoxylin stain.

Area G: In another area the red staining masses inside the dilated canals are seen to be formed through necrosis of the lining cells of the intracanalicular papillæ. Between the glandular structures we find hyaline connective tissue formations.

Area H: An area near area B shows the connective tissue undergoing myxomatous change.

Area I: Another area consists of connective tissue of myxoid, and at other places of hyaline character. In the myxoid as well as the hyaline areas the connective tissue becomes partly transformed into cartilage. Some of the blood vessels show endarteritic changes. These areas are surrounded by a connective tissue capsule. In their vicinity we find several adenomatous nodules with cystic changes. The yellow, golden pigment described in the epithelial cells of the ducts may also be seen in the connective tissue which surrounds the gland concentrically.

2. Piece of the tumor which had not ulcerated, and had been removed December 5, 1906. This tumor shows

adenomatous structures, resembling the mammary gland, lying in myxoid tissue with various degrees of vacuolization.

3. Nodule removed from the left side (from the original, ulcerated tumor, December 31, 1906). The sections show in the center, degenerated tissue; at the margin we find typical preserved tumor structure.

4. Nodules from the original ulcerated tumor removed January 31, 1907. One nodule shows almost normal mammary gland, some of the alveoli contain colloid material. A lymph gland nearby shows blood pigment and numerous phagocytes, its trabeculæ are thickened. Other sections show the typical tumor with cartilage and myxoid connective tissue in which adenomatous tissue with ducts containing colloid is found. Intracanalicular growth is to be noted compressing some of the ducts; here and there small islands of cartilage are seen developing in the proliferating connective tissue. In some of the blood vessels endarteritic proliferation has taken place.

5. Nodule of tumor through which thread had been pulled on December 5, 1906. Nodule was taken out twenty-six days later, on December 31, 1906. The sections show adenomatous tissue, connective tissue, and cartilage. The tumor has the same structure as in other nodules described above. We find cartilage, myxoid tissue, and the different types of adenomatous tissue with the gradations from almost normal to quite atypical tissue. The tissue is well preserved and shows no necrosis. Whole papillæ seem transformed into masses of golden, pigment cells. Other cells in these papillæ show the vacuolar character, as seen in sebaceous glands. Several rows of the epithelial lining cells have become changed into yellowish stained masses, the nucleus has been pushed to one side and has become pyknotic. Similar cells are scattered through the connective tissue. They probably represent the remains of adenomatous tissue which degenerated. The thread did not produce any proliferative or any other noticeable change in the nodule.

II. Pieces of tumor transplanted into the dog affected with the tumor:

1. One piece of tumor not ulcerated, transplanted subcutaneously October 25, 1906, piece removed forty-one days later on December 5, 1906. The section shows well-preserved adenomatous tissue, and a relatively large amount of myxoid tissue, which is somewhat vacuolar.

2. Piece of nonulcerated tumor had been transplanted into same dog October 25, 1906. On December 5th part of the transplanted piece had been removed. December 31st the rest of the transplanted piece was excised. Sections of the piece taken out December 5th show adenomatous tissue and connective tissue, which is relatively poor in nuclei. In some places normal tissue of the mammary gland was preserved. The excision of a piece of the transplanted nodule did not produce any proliferative change.

3. Piece transplanted into dog originally affected with the tumor October 25, 1906, removed December 31, 1906. The sections show typical epithelial tumor tissue, and the structures of the normal mammary gland. Besides there are present nodules showing a transitional tissue. Inside the tubules we find colloid. The connective tissue shows the changes described in the original tumor. No necroses are found.

4. Piece of tumor transplanted into same dog October 25, 1906. One part of transplanted tumor removed December 5, 1906. On December 31st, the remaining part removed shows, on microscopical examination, mostly epithelial structures of the ordinary mammary gland. Some areas of the glandular tissue show intracanalicular growth. There are, besides, large spaces filled with blood and with intracanalicular ingrowths, lined with flat cells. It is doubtful whether the latter structures represent blood vessels or glands; they are probably blood vessels. The connective tissue between the glandular structures is not rich in cells. A considerable part of the tissue has been destroyed by hemorrhage.

5. Piece of tumor transplanted in original dog October 25th, removed December 31, 1906. The sections show the

structure of the original tumor, with colloid material in the dilated tubules. In certain places, intracanalicular papillary ingrowths can be seen in the glandular ducts; several layers of epithelial cells are present in some ducts. Small areas of myxoid connective tissue are found in the tumor. No pigment is found. This piece had remained stationary after transplantation.*

6. Piece of tumor transplanted into original dog on December 31st, had somewhat decreased in size. Removed on January 28, 1907. No necroses are visible. The glandular and connective tissues are both alive. The epithelial parts show partly the structure of the ordinary mammary gland. Stratified epithelium is seen in some of the ducts, and epithelial pearls are found to originate through hyaline change of the cells. Some cells in the layer of stratified epithelium contain yellow colloid bodies. The pigment cells show a decrease in the amount of pigment. The connective tissue shows hyaline change in the center of the section.

7. Another nodule transplanted twice, on October 25th and December 5th, into the same dog; taken out for microscopic examination December 31st, it showed in the periphery living tumor tissue, but in the center it was necrotic. The tumor is made up chiefly of adenomatous structures. In the dense connective tissue few normal glands are found.

III. Piece of tumor transplanted into another dog, December 31st, removed twelve days later.

Microscopic examination shows necrotic tumor tissue and ordinary granulation tissue. The transplanted tumor nodule had not survived.

Conclusions drawn from microscopical Examination.

The tumor used for these experiments was one of the mixed tumors of the mammary gland, which are especially

* Through accident a few pieces transplanted into the original dog had possibly been interchanged during the course of preparation for microscopical examination. This does not affect the conclusions, as the microscopical character of these sections was similar.

common in dogs. According to the hypothesis of Wilms, whose views on the origin of complicated tumors of the type of ours have been accepted by many writers, the morphological character of the tumor is to be explained as follows: At a very early embryonic stage, soon after the three-germ layers have been differentiated, a part of the ectoderm, which is to give rise to the mammary gland, and a part of the mesenchyme are detached from their natural connections and later on begin to grow. The epithelial tissue differentiates in the course of its proliferation into cylindrical and into stratified squamous, in a way similar to the differentiation which occurs in the development of a normal gland, the mesenchyme forming the various kinds of connective tissue. According to this hypothesis, we should expect to find the growth of quite undifferentiated ectoderm undergoing gradual differentiation. In our sections we find a different picture. In different pieces of tumor, in transplanted and nontransplanted, we find, scattered over the tumor, areas of normal or almost normal, mammary gland tissue, and at other places areas of mammary gland tissue transformed into adenomatous tissue; frequently different contiguous areas showed gradual transitions from the normal to the abnormal. The most plausible explanation is, therefore, that the starting point of our growth is the fully developed mammary gland. Under the influence of an unknown stimulus, the tissue of the mammary gland begins to proliferate, and forms gradually atypical tissue. Various intermediate stages are present, and almost identical changes are found in different pieces. In one nodule we saw the formation of stratified epithelium and of epithelial pearls. These formations, however, cannot be interpreted in the manner advocated by Wilms. The mammary gland ducts themselves form the stratified epithelium. It is not a question of undifferentiated epithelium developing into stratified epithelium. The typical yellow pigment in some of the cells of these epithelial formations indicate that we are dealing with fully developed mammary gland tissue. It is unlikely that the changes underlying this process of pearl formation are

identical with the normal process of keratinization. The connective tissue structures develop partly in close relation with the glandular structures, at other places they form separate nodules. In some of these nodules epithelial formations were probably present at a previous period. At least this is an interpretation which might be given to the presence of the yellow pigment in the connective tissue; the epithelium having degenerated the pigment remained in the connective tissue. The presence of extensive areas of myxoid and hyaline connective tissue and of cartilage does not force us to assume an embryonic origin of such tumors, especially since the transformation of connective tissue into bone has been found to take place under various conditions in adult animals. There is probably a stimulus acting on both epithelial and connective tissues of the mammary gland; whether or not the stimulus is the same for both tissues cannot be said; neither can it be stated on which tissue the stimulus was acting primarily if the stimulus acting on both tissues was identical. But even, if we grant that an error in development is responsible for the fact that different structures are affected simultaneously, the explanation given by Wilms would have to be modified in accordance with our findings.

Tumors were found, simultaneously, on the right and left side of the dog. Have we here to do with a metastasis or with two separate tumors? Microscopically both tumors have the same structure. It seems more likely that the mammary glands on both sides of the body were affected independently of each other. Otherwise, we should expect to see metastases elsewhere; but none could be recognized during the twelve months in which the animal was under observation.

Nowhere did we see any sign of active cell multiplication. If any growth took place in the last year it must have been very slow indeed. Without any very active cell growth the tissues were found, on section, to be alive and healthy, epithelial as well as connective tissues.

A very important result of the microscopic examination is

the observation that no marked difference exists in the character of the pieces of the original tumors, which were left untouched, and those which were transplanted into the same animal. They all showed the same degree of vitality, without any sign of degeneration. In one piece of the original nontransplanted tumor some central necrosis was found. Central necrosis was found likewise in one tumor nodule which had been transplanted twice. In one piece, from which after transplantation a portion had been cut out, some hemorrhage was noted. On the whole, however, the remarkable fact remains that after transplantation into the same animal no central necrosis took place. This was true of both epithelial and connective tissue structures. It is the more remarkable, as we find after transplanting sarcoma, as well as carcinoma, in mice and rats that invariably a central necrosis takes place, with the exception of those cases in which very small pieces have been transplanted, which latter, as one⁵ of us pointed out six years ago, may remain alive entire.

These observations, however, agree perfectly with the observations published in the summer of 1902, by one of us, in which it was shown that after retransplanting a mammary adenoma of a white rat the pieces transplanted into the same animal would remain alive without any central necrosis being present.

Another result of our microscopical examination is the observation that those means, which in other varieties of tumors caused active cell proliferation, as for instance, pulling a thread through a tumor, or excising a portion of the transplanted nodule, or retransplanting a piece of tumor, were without any effect whatever upon the tumor cells. No change was produced in the condition of the remaining tumor tissue, neither of the epithelial nor of the connective tissue elements.

In very marked contrast to the excellent condition of the pieces transplanted into the same animal in which the tumor had originated, stands the fate of the pieces transplanted into other animals. As we saw, they all disappeared in a

relatively short time. Soon after transplantation we found, on microscopical examination, that the transplanted pieces had become necrotic, and the connective tissue grew into the necrotic area. The same changes took place, therefore, here, as in other cases of tumors unsuccessfully transplanted.

DISCUSSION OF RESULTS AND CONCLUSIONS.

I. We wish to consider the experiments recorded here, in connection with others already mentioned, made with a mammary adenoma and with experiments on the production of benign deciduomata in a guinea-pig.⁷

The essential facts which have to be considered in this connection are the following:

(1.) A mammary adenoma of the rat remains alive when it is transplanted into the same individual in which the tumor has taken its origin. Not only the peripheral parts of the tumor remain alive, but the central parts as well. Pieces transplanted into other rats become entirely necrotic or, at the best, some peripheral parts were found to remain alive and even to grow after transplantation, the central parts becoming necrotic. This applies to the epithelial as well as the connective tissue.

(2.) In a mixed tumor of a dog, pieces of tumor, transplanted at various times into the original tumor dog, not only remain alive in the peripheral parts, but in the central parts as well, although they do not show any signs of growth. This applies to the epithelial as well as to the connective tissue structures. Transplanted into other dogs the pieces become entirely necrotic.

(3.) At a time when in the first experiment the original tumor rat was pregnant, not only her own mammary gland began to grow, but also the transplanted adenomatous structure as well as the original tumor assumed a very rapid growth; no milk, however, being produced in the tumors. This growth stopped, simultaneously, in both mammary gland and tumors.

(4.) About four to six days after copulation, when normally the decidua is beginning to be formed under the

influence of the ovum which penetrates the epithelial covering of the uterine mucosa, non-specific stimuli may cause the production of decidual nodules without contact of an ovum having previously taken place, and without a pregnancy existing at the time of the development of the decidua.

(5.) After a time the deciduomata become spontaneously necrotic in their entirety.

(6.) In the course of transplantations of tumors in white rats or mice and in the case of successful transplantation into other individuals of the same species, the transplanted piece becomes necrotic in the center, only the peripheral parts remaining alive and giving origin to the tumor. In cases where very small pieces are transplanted the whole piece may remain alive. This was found in the case of sarcoma (Loeb),⁷ and afterwards by Jensen and others, in the case of the adenosarcoma of mice.

(7.) If we transplant normal or regenerating skin it is especially the marginal epithelium which remains alive after transplantation. The centrally situated epithelial tissue can remain alive in such cases in which the epithelial tissue is covered only by a thin layer of connective tissue. If considerable connective tissue or cartilage covers the epithelium of the skin it becomes necrotic. The necrosis is caused by the lack of substances necessary for the life of the epithelial cells, the connective tissue preventing access to these cells.

These facts enable us to draw certain conclusions and to define new problems:

(a.) The inaccessibility of the central parts to the substances necessary for the metabolism of the cells might be thought to be the cause of the central necrosis found after the transplantation of mouse and rat tumors. That such a factor is of importance is plainly shown by the results of transplanting epithelium. Our experiments, however, show that the problem is a more complex one.

Pieces of the mammary tumors of the rat or dog, of such a size that a central necrosis would have taken place if an equally large piece of sarcoma of the rat or adenocarcinoma of the mouse had been successfully transplanted into another

individual of the same species, remain alive entire, if they are transplanted into the same animal in which the tumor has originated; they become entirely necrotic or show a very extensive area of central necrosis if transplanted into another individual of the same species. The body fluids in different individuals of the same species are, therefore, different. Is this difference between different individuals confined to tumors, or does it apply to other tissues and organs as well? No systematic experiments on this question seem to have been made, but from certain occasional statements in the literature it is probable that certain organs, as the ovaries, behave differently according to whether they have been transplanted into the same or into other individuals (Knauer). But we have to delay our final conclusions on this question until experiments which are under way shall have been concluded.

Furthermore, we cannot state definitely whether the individual in which the tumor had developed furnishes a certain substance favorable to the tumor cells, or whether in this individual certain injurious substances are absent which are present in other individuals of the same species. In a general way it can, however, be stated that the composition of the body fluids in the individual in which the tumor was growing differs in some respects radically from the body fluids of other individuals of the same species, and that the former is much more favorable to the transplanted cells.

Our experiments, carried out with the mammary tumor of the rat, were complicated by a pregnancy that made itself felt very soon after transplantation of the tumor, and caused the tumor tissue, which must have remained alive immediately after transplantation, to assume somewhat later an increased growth. The new experiments with the mammary tumor of the dog form, therefore, a welcome supplement to the former experiment in so far as they show, without any doubt, and without the presence of any complicating factor, that the cells remained alive after transplantation into the same individual without any cell proliferation taking place.

(b.) The experiments mentioned under (1) and (2)

show that we have to differentiate substances which permit tumor cells to live, and substances which permit them to grow. Here we have only to deal with substances which permit them to live; they are present in the individual in which the tumor grew, they are absent in all other individuals tested.

This raises a new, interesting problem: How far substances which permit the life of tumor cells may be identical with the substances causing them to grow. Our experiments give some indication in this direction. These results, however, will have to be tested and extended in further investigations.

The experiment described under (3) shows that these two substances can be different. At first there was present only that substance or mixture of substances which permitted the tissue to live, but very soon during pregnancy a new substance or new substances were added that caused the living cells to proliferate. After some time the production of this substance stopped, and then the growth ceased to continue. This interpretation coincides with the interpretation given by one of us in a former communication.

The experiments recorded in (4) and (5) render it very likely that under certain conditions the substances causing the proliferation of tissues are identical with those substances which permit them to retain their vitality. The deciduomata became necrotic soon after they had ceased to grow. In all probability their growth depends upon a combination of two conditions: (1) upon the presence of a general predisposing factor independent of local conditions in the uterus, which probably consists in the presence of a chemical substance; (2) upon a local stimulus to grow.

Inasmuch as the tumor pieces transplanted into the original animals behaved like the original nontransplanted tumors in regard to growth, we can, with a great degree of probability, assume that if the composition of the body fluid enables the tumor cells to live, the latter are also able to grow, provided the tumor cells themselves are the carriers of a stimulus to growth which is localized in or near the transplanted tumor cells. In our case of transplantation of

the mammary tumor of the dog and rat, we found a quiescent condition of the cells, because they carried with them only a weak localized stimulus. The presence of such a localized stimulus is necessary for the permanent growth of tumors after transplantation. A transitory growth may, however, be caused, as in experiment No. 3, by substances circulating in the organism in which the cells are living.

It follows from these considerations that for the successful transplantation of tumors at least two sets of conditions must be present: (1) a stimulus to grow, localized in the tumor cells themselves; (2) the presence of substances permitting the life of the transplanted cells in the organism into which the tumor cells are transplanted; (3) there is, perhaps, also necessary a substance like that mentioned in experiment No. 3 favoring the growth of tumor cells. The necessity for the presence of such a third substance might depend on the strength of the localized stimulus present in the tumor itself. If this stimulus is very strong, only a very small quantity of the substance mentioned in No. 3 might be necessary, or it might be dispensed with entirely. If that localized stimulus is very strong, even substance No. 2 might be necessary only in very small quantities, or transitory growth might take place without it. If substances are present in the host-organism that are directly injurious to the tumor cells, the localized factor No. 1 and the substance No. 3 must be present in a correspondingly large quantity if a growth shall take place. To this may be added differences which may originally exist in the conditions necessary for the life and growth of that particular tissue which became converted into tumor, and we may in this way account for the different behavior of different tumors after transplantation. Although a part of these considerations are hypothetical, others are not, and they may help us in a further experimental analysis of the conditions upon which the outcome of the transplantation of tumors depends.

II. In the former experiments of one of us^s it was found that a tumor can be caused to assume an increased growth by certain indifferent stimuli, as for instance pulling

a thread through a quiescent part of the tumor or cutting out a portion of the growth. The energy of growth can also be stimulated up to a certain maximum by retransplanting a piece of tumor.

Similar results were obtained in the case of the tumor found in the submaxillary gland of a Japanese mouse.⁸ Sticker,⁹ in his work on lymphosarcoma, obtained similar results. Does this readiness to respond to such stimuli apply to all tumors? Our experiments on the mammary tumor of the dog show that this is not the case. None of these means mentioned above were capable of causing increased proliferation of the tumor cells, although such attempts were made repeatedly.

We may, therefore, conclude that different tumors differ in regard to the readiness with which they respond to certain non-specific stimuli.

Tumors differ therefore:

1. As to whether they are transplantable or nontransplantable.
2. As to whether they are transitory or permanently growing tumors.
3. As to whether they are rapidly or weakly growing tumors.
4. As to whether or not they are infiltrating tumors, and whether they are metastasizing or non-metastasizing.

To this may be added:

5. As to whether they are labile or stable, according to whether or not they respond to the above-named stimuli.

How far these different qualities appear in combination has still to be investigated. Tumors, *e.g.*, which are growing very weakly in the first generation, may be very easily transplantable.

If we summarize the results obtained in regard to the inoculability of tumors we can perhaps divide the different tumors into three groups:

- (1.) The lymphosarcoma of dogs has so far been shown to possess the widest range of inoculability: it can be

transplanted not only into the dog but into other species like the fox.

(2.) The sarcoma of white rats and the adenocarcinoma of white mice can be transplanted into white rats and into white mice respectively, but also, though with much greater difficulty, into hybrids, between white and gray (wild) rats, in the case of sarcoma, and into gray mice in the case of adenocarcinoma of white mice.

(3.) A third class of tumors, such as the adenocarcinoma found in the Japanese mouse, cannot be transplanted into white mice; it shows therefore the greatest sensitiveness in regard to species.

In a certain small percentage of cases, according to the findings of several investigators, the adenocarcinoma of white mice can be transplanted into gray mice, but not into the other strains of white mice. In a similar way the sarcoma of the rat could be transplanted into hybrids, between white and gray rats. Certain white rats, however, seem to be refractory. Certain white mice and rats may, therefore, differ more radically in regard to that composition of their body fluid which permits the tumor to grow than certain white mice or rats differ from gray mice or gray rats. The reverse is found in the case of the tumor of the Japanese mouse; it could be transplanted into all Japanese mice (although it was at first a slowly growing tumor) but could not be transplanted into white mice.

(4.) The last class of tumors, and it includes probably the largest number of tumors, comprises all those which, under the ordinary conditions of work, cannot be inoculated into other animals of the same species. From the experiments made in the transplantation of human tumors, we may conclude that at least many of the tumors in man can only be inoculated into the same individual in which the original tumor was developing.

This applies probably to the majority of tumors found in man and in lower animals. (A summary of the results is given at the end of the chapter on the experimental part

and on the microscopical examination. Conclusions of a more general character are stated in the last chapter.)

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